REMARKS/ARGUMENTS

Claims 1-4, 9, 75-127, 140 and 143-146 remain herein. Claims 5-8, 10-42, 58-74, 128-139, 141 and 142 are canceled hereby, without prejudice or disclaimer. New claims 147-214 are added hereby. Thus, claims 1-4, 9, 75-127, 140 and 143-214 are pending herein. Claims 9 and 75-127 have been withdrawn from consideration by the U.S. PTO.

Claims 10-42, 58-74, 128-139, 141 and 142, many of which had previously been withdrawn from consideration by the U.S. PTO, are canceled at this stage solely to avoid paying additional fees for the new independent claims submitted herewith, and so their cancellation should not be interpreted as creating any estoppel.

Claims 1-8 were rejected under 35 U.S.C. §102(e) over U.S. Patent No. 5,795,748 (Cottingham '748). In addition, claims 1-8 were rejected under 35 U.S.C. §102(e) over U.S. Patent No. 5,948,673 (Cottingham '673).

Claims 1-4 each recite that the reaction product of amplification of a single molecule of the target nucleic acid comprises a clonal amplified product having a detectable concentration within a portion of the sample chamber after a single round of amplification.

Cottingham '748 discloses an apparatus for carrying out a homogeneous nucleic acid amplification and nucleic acid assay on a liquid biological sample comprises a sample well and an optical window element which is received in the sample well. Opposed, spaced-apart surfaces of the optical window element and sample well define a capillary chamber into which a liquid biological sample is drawn by capillary force. By spreading the liquid biological sample into a thin film within the capillary chamber, head space is eliminated, heat transfer to the sample is maximized, and a large optical target is achieved to facilitate the detection step of the assay. The disclosed apparatus is particularly suited for use with homogeneous nucleic acid amplification and fluorescence polarization assays. Cottingham

'748 discloses that an object is to provide a low-volume reaction device that has virtually no head space, does not require that external heaters be provided on top of the device, and is not subject to evaporation and condensation of the liquid biological sample contained within the device.

Cottingham '748 discloses that FIGS. 5A and 5B are sectional views illustrating the internal configuration of the sample well assembly 22, with the seal 18 shown removed in FIG. 5A and fully installed in FIG. 5B. According to Cottingham '748, as best seen in FIG. 5A, the optical window element 20 is held by the notched ribs 26 in a parallel relationship with the bottom wall 32 of the sample well 14, with a uniform gap (preferably about 0.020 inch in height). In the preferred embodiment, Cottingham '748 discloses, the sample well 14 is formed with an inside diameter of approximately 0.290 inch, and the optical window element 20 is formed with an outside diameter of approximately 0.250 inch. Cottingham '748 discloses that the liquid biological sample 60 substantially fills the capillary chamber 54, which has a volume of about 20 microliters.

Cottingham '673 discloses a DNA amplification and homogeneous DNA probe assay device which includes a multiplicity of discrete sample cells in a flat "card" format, with each sample cell containing the reagents necessary for both DNA amplification and homogeneous DNA probe assay. Referring to Fig. 1, the card 20 contains a rectangular array of discrete sample cells 22, spaced evenly across the length and width of the card. Each sample cell 22 includes a closed sample chamber 24 (the top wall of which is transparent) for receiving a liquid biological sample, an open sample port26 which communicates with the sample chamber 24, and an air vent 28 which also communicates with the sample chamber 24. According to Cottingham '673, the volume of the liquid biological sample will typically be very small (about 20 microliters). Sealing strips 32 are provided for sealing the sample ports

26 and air vents 28 of the sample chambers 24 after liquid biological samples have been introduced into the sample cells 22. According to Cottingham '673, the sealing strips prevent the release of DNA amplicons from the sample cells 22.

As discussed below, neither Cottingham '748 nor Cottingham '673 discloses or suggests a method for detecting a target nucleic acid molecule in a sample, in which the reaction product of amplification of a single molecule of the target nucleic acid comprises a clonal amplified product having a detectable concentration within a portion of the sample chamber after a single round of amplification, as recited in claims 1-4.

It is well known that the larger a sample volume, the larger the number of target molecules needed in order to be detectable. It follows that the larger the sample volume, the larger the number of cycles that would be needed in order to increase the concentration of a target molecule where only a single molecule of the target molecule was present in the original sample. It is also well known, however, that as the number of cycles during a single round of amplification increases, artifactual amplicons derived from the primers (so called "primer-dimers") begin to appear (see the specification, page 6, line 8 - page 7, line 14. It is further well known that the appearance of such artifactual amplicons results in the molecules detected as contributing to the concentration of target molecule being ***non-clonal***.

In each of Cottingham '748 and Cottingham '673, each sample volume is on the order of 20 microliters. With such a large sample volume, if only a single molecule of the target nucleic acid were present, the reaction product of amplification of the target nucleic acid would not reach a detectable concentration in a single round of amplification, if at all, until after a number of amplification cycles which would generate an enormous amount of artifactual amplicons, whereby any detectable concentration of the target nucleic acid would be far from clonal (n.b., claims 1-4 recite "the reaction product of amplification of a single

molecule of the target nucleic acid comprises a ***clonal*** amplified product having a detectable concentration within a portion of the sample chamber after a single round of amplification).

Accordingly, claims 1-4, and the claims which depend therefrom (namely, claims 132-146), are patentable over Cottingham '748, Cottingham '673 or any valid combination thereof.

Claims 132-146 were rejected under 35 U.S.C. §103(a) over Cottingham '748 or over Cottingham '673. Claims 132-146 each depend from claim 1, and are therefore patentable over Cottingham '748 or and Cottingham '673 for the same reasons as discussed above regarding claim 1. In addition, it is respectfully noted that Cottingham '748 and Cottingham '673 provide no indication that sample size and/or sample chamber size are optimizable, or that they should be (and could be) reduced to the levels claimed. Cottingham '748 and Cottingham '673 disclose no recognition of the advantages obtained in accordance with the present invention, and so one would not be looking for those advantages in any attempt to optimize sample size and/or sample chamber size if for some reason an attempt was being made to optimize such size(s).

Accordingly, it is respectfully requested that the U.S. PTO reconsider and withdraw these rejections.

Favorable consideration of new claims 147-150 is respectfully requested. Claims 147 and 149 each recite a method comprising loading a sample into a sample chamber which includes structure which limits diffusion of a reaction product of amplification of the target nucleic acid within a portion of the sample chamber, and subjecting the sample to at least a first round of amplification such that a reaction product of the amplification of a single molecule of the target nucleic acid comprises a detectable concentration within said portion of the sample chamber after the first round of amplification. Claims 148 and 150 each recite

a method comprising loading a sample into a sample chamber which includes means for limiting diffusion of a reaction product of amplification of the target nucleic acid within a portion of the sample chamber, and subjecting the sample to at least a first round of amplification such that a reaction product of the amplification of a single molecule of the target nucleic acid comprises a detectable concentration within said portion of the sample chamber after the first round of amplification.

As discussed below, neither Cottingham '748 nor Cottingham '673 discloses or suggests a method comprising loading a sample into a sample chamber which includes structure which limits (and/or means for limiting) diffusion of a reaction product of amplification of the target nucleic acid within a portion of a sample chamber, and subjecting the sample to at least a first round of amplification such that a reaction product of the amplification of a single molecule of the target nucleic acid comprises a detectable concentration within said portion of the sample chamber after the first round of amplification.

In each of Cottingham '748 and Cottingham '673, a sample volume on the order of 20 microliters is loaded into each of the sample chambers. Neither Cottingham '748 nor Cottingham '673 provides any structure or means which would limit diffusion or reaction product of amplification within any portion of the sample chamber, nor does either reference disclose or suggest any reason for providing any such diffusion limiting. In the context of the present invention, limiting such diffusion is important in making it possible to detect the reaction product of a single molecule of target nucleic acid in a single round of amplification (while minimizing the number of cycles needed).

Accordingly, claims 147-150 are patentable over Cottingham '748, Cottingham '673 or any valid combination thereof.

In view of the above, claims 1-4 and 132-151 are in condition for allowance.

If the Examiner believes that contact with Applicant's attorney would be advantageous toward the disposition of this case, the Examiner is herein requested to call Applicant's attorney at the phone number noted below.

The Commissioner is hereby authorized to charge any additional fees associated with this communication or credit any overpayment to Deposit Account No. 50-1446.

Respectfully submitted,

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